WS15.1 Hyperosmolar saline causes detachment of cystic fibrosis mucus

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Objectives: Cystic fibrosis (CF) is caused by mutations in the gene encoding the CFTR, an ion channel transporting chloride and bicarbonate ions. Patients with CF have stagnant mucus, resulting in insufficient mucus clearance, bacterial infections and lung damage. The main constituent of mucus is secreted, gel forming mucins, stored in goblet cells. Mucus is densely packed in goblet cell granulae, the packing being tightly controlled by the N-terminal part of the mucin and requires calcium ions, as we have shown for the intestinal MUC2 mucin. Properties of mucus expansion in the ileum would presumably be general for all gel forming, secreted mucins, such as MUC5AC in the airways. To increase mucociliary clearance, the usefulness of hypertonic saline (HS) has been investigated in patients with CF at least since 1994, but the mechanism of action remains undetermined.
Methods: Using an explant system, we have previously shown that the mucus of the small intestine is easily removable in the WT mouse ileum, whereas it is attached to the epithelium in mice without a functional CFTR channel (CftrΔF508). This phenotype could be reverted to a non-attached phenotype by apical solutions containing above 100 mM bicarbonate.
Results: In the CF mouse ileum, hypertonic saline between 1.75 and 5% detached the mucus from the epithelium. Between 2 and 5% the mucus also expanded over 1 hour. At 2%, HS did not make CftrΔF508 ileal mucus penetrable to beads the size of bacteria.
Conclusion: HS, a common CF therapy, was effective in detaching the mucus, indicating a mechanism of action. Further research is required to identify the molecular identity of the mucus attachment in CF.

WS15.2 The secreted mucus proteome in ileum of a cystic fibrosis mouse model

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Objectives: The gastrointestinal epithelium is protected by mucus that, under physiological conditions, is continually secreted and renewed. In small intestine the normal mucus is not attached and easily removed; however, a mouse model of CF (CftrΔ508) displays attached mucus in the ileum, leading to some of the gastrointestinal symptoms of the disease. Thus we characterized the proteome of the ileal mucus in CF and normal mice, to find differentially expressed proteins that could be responsible for the observed phenotypes.
Methods: Four C57BL/6 CftrΔ508 (CF) mice and four controls were sacrificed, and the ileum was dissected and mounted in an Ussing-type chamber under physiological conditions. Secretion was stimulated with 10 μM carbachol and prostaglandin E2, mucus was collected and solubilized, and proteins digested. The obtained peptides were analyzed by tandem mass spectrometry, and the mucus proteins identified and quantified.
Results: The mucus proteome of CF and normal mice showed 68% similarity. Almost 600 proteins were identified in both strains, 80 were detectable only in CF mice, and more than 300 appeared only in healthy mice. When we examined the individual levels of the shared proteins, a striking difference was found in the proteolytic enzyme meprin beta that was decreased 80% in the CF mucus. Other known mucus proteins (as Muc2, Clca1 and Fcgbp) revealed increases of 50 to 100% in CF mucus.
Conclusion: The CF mucus proteome contains up to 2-fold the amount of typical mucus constituents than the normal mucus. The lower levels of the enzyme meprin beta could contribute to the attached CF mucus.

WS15.3a Digestibility of macro minerals (prececal and total tract) in growing pancreatic duct ligated piglets − used as a model for exocrine pancreatic insufficiency in children

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Objectives: CF patients often suffer from exocrine pancreatic insufficiency (EPI). Although lung infections are the major cause of death, maldigestion and malabsorption of nutrients is of special interest as BMI directly affects survival rate. This study was focussed on the effects of EPI on apparent digestibility (app. dig.) of macro minerals in juvenile pigs.
Methods: The study was performed in 9 piglets. In 4 of these pigs the pancreatic duct was ligated (PL) at 7 weeks of age, the other 5 pigs underwent a sham OP and served as controls (C). None of the PL-pigs received pancreatic enzymes. A complete diet (g per kg dry matter: 373 starch, 115 crude fat, 203 crude protein; 12.1 Ca, 5.97 P, 1.98 Mg, 1.72 Na, 7.46 K, 4.39 Cl) was fed restrictively; except for the last two weeks (ad libitum feeding). Chromium oxide was used to calculate app. dig. of macro minerals by the marker method. Animals were euthanized at the age of 26 weeks. Ingesta from the rectum were taken for analysis (using standard methods).
Results: There was no effect of EPI on app. dig. of Ca, P and Mg, but EPI caused a marked reduction of app. dig. of Na (C: 75.0±15.2; PL: 9.78±25.6), K (C: 82.6±3.70; PL: 53.4±12.7) and Cl (C: 94.3±1.39; PL: 86.4±12.7) over the entire gastrointestinal tract.
Conclusion: As app. dig. of Ca and P did not differ between PL- and C-pigs the lower bone density in growing pigs seen previously must be a result of other factors (e.g. IGF-1). The lower app. dig. of electrolytes in PL-pigs is worth mentioning especially when considering that CF patients have increased losses of salt via sweat due to dysfunction of the CFTR receptor.

WS15.3b Pancreatic duct ligated piglets − used as a model for children: effects of exocrine pancreatic insufficiency (EPI) and pancreatic enzyme replacement therapy on different parameters of growth

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Objectives: The pancreatic duct ligated pig is an established model of EPI in humans – but studies in juveniles are rare. This study was conducted in growing pigs with experimentally induced EPI to test its effects and that of enzyme supplementation on growth.
Methods: EPI was induced experimentally in 8 of 12 piglets by ligation of the pancreatic duct (PL) at the age of 8 weeks. PL-pigs were divided into two groups: **PL** (no enzyme supplementation) and **PL+enz** (porcine pancreatin, Creon®; 9.9 g (~518,700 IU lipase; 29,660 IU protease; 484,526 IU)/kg feed). The other pigs (n = 4) were sham operated and served as control (C). Animals were housed individually and fed identical amounts of the diet. Last week before dissection the diet was given *ad libitum*. Every 2nd week vitamins (A, D, E, K, C, B) were supplemented via intramuscular injection. Animals were euthanized at an age of 19 weeks. Empty body weight was calculated as body weight (bw) minus mass of GIT.
Results: EPI resulted in a markedly lower bw (C: 79.7; PL: 51.3; PL+enz: 62.9 kg) and furthermore in a much lower empty bw (C: 73.2; PL: 40.5; PL+enz: 55.4) caused by a higher mass of the GIT). PL-pigs were shorter in length and had a lower perimeter of metacarpus and breast – but abdomen perimeter was similar in all groups.
Conclusion: Even enzyme supplementation led to a distinct increase in bw and length, the levels of healthy controls were not reached, indicating that a higher dosage is necessary or that the status of some nutrients could not be normalized (e.g. fat soluble vitamins). The higher mass of GIT might cause an overestimation of nutritional status and BMI in such patients.